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EXAMINER	
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ART UNIT	PAPER NUMBER
1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/759,416	ANSARI, ASEEM Z.	
	Examiner	Art Unit	
	Laura McGillem	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 January 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-38* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-38* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *16 January 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *4/8/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Priority

It is noted that this Application received priority benefit of Provisional Application No. 60/759,416, filed 1/16/2003.

Claims 1-38 are under examination.

Claim Objections

Claims 25-26 are objected to because of the following informalities: there is a duplication of the word "step". Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-12 and 22-30 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-12 and 22-30 are vague and indefinite because they recite the phrase "artificial regulatory factors" and the metes and bounds of what constitutes an "artificial" factor are not clear. The specification does not provide a definition of artificial regulatory factor. As the claim is written, the phrase is vague because the skilled artisan would not know how a regulatory factor would need to be artificial in order to meet the claim limitation. For example, does the claim limitation require that the regulatory factor be

made artificially by peptide synthesis; does it require a factor that would not normally be a regulatory factor; or does it require that the regulatory factor be one not found in nature? The specification does not designate regulatory factors that would be found in nature so that the skilled artisan would know which would be artificial.

Claims 12, 21, 30, 34 and 38 are vague and indefinite because they recite the phrase "entropically destabilized" and the metes and bounds what constitutes an "entropically destabilized" linker are not clear. The metes and bounds of an entropically stable linker are not clear, so that the skilled artisan would know whether the linker is "entropically destabilized" in order to meet the limitation of the claims.

Claims 2-11 and 21-29 are indefinite insofar as they are dependent on an indefinite claim.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 1-10, 13-19 and 31-32 are rejected under 35 U.S.C. 102(b) as being anticipated by Ansari et al (2001, Chem. Biol. Vol. 8, pages 583-592, online pub. 5/8/2001).

Ansari et al teach an artificial transcriptional activator with a polyamide, non-protein DNA binding motif composed of heterocyclic residues that bind to the minor groove of DNA (see page 584, left column, 2nd paragraph, for example). Ansari et al teach that the polyamide, non-protein DNA binding motif is attached to a flexible polyether linker (i.e. oxygen atoms connected to two alkyl groups) (see page 584, left column, 3rd paragraph, and page 585, Fig 2, for example). Ansari et al teach that the linker attaches the polyamide, non-protein DNA binding motif to an activating region (AH) of approximately 20 residues to form a conjugate (PA-1L-AH) (see page 584, left column, 3rd paragraph, for example). Ansari et al teach that the PA-1L-AH conjugate motif functions in a cell free system, and Ansari et al modified this conjugate motif to try to upregulate transcription. Ansari et al teach that the identity of the activating region was varied by size, identity and point of attachment to the polyamide. Ansari et al teach that the three activating regions tested were identified as AH, VP1 and VP2 (one or two tandem repeats of an eight amino acid sequence derived from the viral activator VP16) (see page 584, Table 1 and page 587, left column, for example), which reads on a test compound, bound to a linker moiety covalently bonded to a polyamide anchor moiety. Ansari et al teach a plasmid vector with three cognate palindromic sequences upstream of a AdML TATA box, which is upstream of a G-less cassette (see page 591, left column, 2nd paragraph), which reads on an isolated target nucleic acid that defines one

and only one binding site for a regulatory factor. Ansari et al teach that all of the varied conjugates bound to the cognate sites upstream from the AdML promoter (see page 586, left column).

Ansari et al teach that the activating region is thought to bind to components of the transcriptional machinery that associate with RNA polymerase known as the RNA polymerase II holoenzyme (see page 583, right column). Ansari et al teach that DNAase footprinting titrations under *in vitro* transcription conditions revealed that substituting the AH activator with VP2 increases the transcriptional activation strength of the conjugate over substitution with VP1 (see page 588, paragraph 2.4, for example).

The instant specification broadly defines aptamer as a linker moiety that is dimensioned and configured to bind specifically with a small-molecule binding partner. Ansari et al teach that the linker portion of the conjugate has a critical role in facilitating the projection of the activating domain away from the target DNA for productive interaction with elements of the transcriptional machinery. Ansari et al teach experiments in which the polyether linker had been conjugated at the C-terminus of the polyamide region, but that conjugation at an internal pyrrole residue is also effective (see page 587, left column, 2nd paragraph and page 589, right column, for example), which reads on a linker moiety that has been configured to associate with a binding partner. Ansari et al teach a linker that is 36 atoms long and a linker that is 12 atoms long (see page 585, Figure 2 legend). Ansari et al teach that the activation domains recruit elements of the endogenous transcriptional machinery to the promoter proximal to where the activator protein is bound to DNA (see page 583, left column, for example),

which reads on a method of identifying a test compound (i.e. activating region) that modulate binding of natural or artificial regulatory factors to corresponding double stranded nucleic acid binding sites comprising providing a nucleic acid target (i.e. nucleic acid sequence comprising DNA upstream from the AdML promoter) and an anchor moiety (polyamide DNA binding motif) covalently bonded to a flexible polyether linker covalently bonded to a test compound (VP1, VP2 or AH). It reads on contacting the conjugate-bound DNA sequence comprising an AdML promoter with transcriptional machinery and determining whether binding of a transcriptional regulatory factor of the transcriptional machinery is modulated by the presence of a test compound. The composition taught by Ansari et al also anticipates a kit and composition of matter comprising an isolated nucleic acid target that defines a binding site for a regulatory factor, a covalently bonded an anchor moiety, a linker moiety covalently bonded to the anchor moiety, and a test compound conjugated to the linker moiety wherein the linker is an aptamer.

Ansari et al teach attempts to generate synthetic transcriptional modulators by modifying or replacing the activation domain, the linker domain and the DNA binding motif (i.e. anchor motif). Ansari et al teach that attachment of a triplex forming oligonucleotide to a transcriptional activating region upregulated gene expression (see page 584, left column, 2nd paragraph, for example), which reads on the anchor moiety as a major groove-binding/triple helix forming oligonucleotide.

Claims 22-28 are rejected under 35 U.S.C. 102(b) as being anticipated by Ansari et al (2001, Chem. Biol. Vol. 8, pages 583-592, online pub. 5/8/2001) as evidenced by Sadowski et al (Nature, 1998, Vol. 335, pages 563-564).

Ansari et al teach an artificial transcriptional activator with a polyamide, non-protein DNA binding motif composed of heterocyclic residues that bind to the minor groove of DNA (see page 584, left column, 2nd paragraph, for example). Ansari et al teach that the polyamide, non-protein DNA binding motif is attached to a flexible polyether linker (i.e. oxygen atoms connected to two alkyl groups) (see page 584, left column, 3rd paragraph, and page 585, Fig 2, for example). Ansari et al teach that the linker attaches the polyamide, non-protein DNA binding motif to an activating region of approximately 20 residues to form a conjugate (PA-1L-AH) (see page 584, left column, 3rd paragraph, for example). Ansari et al teach that the PA-1L-AH conjugate motif functions in a cell free system, and Ansari et al modified this conjugate motif to try to upregulate transcription.

Sadowski et al teach a specific transcriptional activator comprising a DNA binding fragment of the yeast activator GAL4 fused to a portion to the HSV protein VP16. Sadowski et al teach that VP16 activates transcription by binding to host encoded protein that recognized DNA sequence in their promoters. Sadowski et al teach that the acidic portion of VP16 is a very strong transcriptional activator in CHO cells (see page 563, right column, 1st paragraph and page 564, left column). Therefore, VP16 is a transcription activator that can modulate binding of natural transcription factor

such as those that are found in cultured CHO cells. Ansari et al teach that some activating regions tested were one or two tandem repeats of an eight amino acid sequence derived from the viral activator VP16 (see page 584, Table 1 and page 587, left column, for example). Since Sadowski et al teach that VP16 is known to modulate binding of natural transcription factors, and Ansari et al use portions of VP16, the VP1 and VP2 activating regions used by Ansari et al anticipate a test compound known to modulate binding of natural transcription factors.

Ansari et al teach a plasmid vector with three cognate palindromic sequences from conjugate binding, upstream of a AdML TATA box, which is upstream of a G-less cassette (see page 591, left column, 2nd paragraph), which reads on an isolated target nucleic acid that defines one and only one binding site for a regulatory factor. Ansari et al teach that all of the varied conjugates bound to the cognate sites upstream from the AdML promoter (see page 586, left column

Ansari et al teach that the activating region is thought to bind to components of the transcriptional machinery that associate with an RNA polymerase II holoenzyme (see page 583, right column). Ansari et al teach that DNAase footprinting titrations under *in vitro* transcription conditions revealed that substituting the AH activator with VP2 increases the transcriptional activation strength of the conjugate over substitution with VP1 (see page 588, paragraph 2.4, for example).

Ansari et al teach attempts to generate synthetic transcriptional modulators by modifying or replacing the activation domain, the linker domain and the DNA binding motif (i.e. anchor motif). Ansari et al teach that attachment of a triplex forming

oligonucleotide to a transcriptional activating region upregulated gene expression (see page 584, left column, 2nd paragraph, for example), which reads on the anchor moiety as a major groove-binding/triple helix forming oligonucleotide.

The instant specification broadly defines aptamer as a linker moiety that is dimensioned and configured to bind specifically with a small-molecule binding partner. Ansari et al teach that the linker portion of the conjugate has a critical role in facilitating the projection of the activating domain away from the target DNA for productive interaction with elements of the transcriptional machinery. Ansari et al teach that a polyether linker can be conjugated at the C-terminus of the polyamide region, but that conjugation at an internal pyrrole residue is also effective (see page 587, left column, 2nd paragraph and page 589, right column, for example), which reads on a linker moiety that has been configured to associate with a binding partner. Ansari et al teach a linker that is 36 atoms long and a linker that is 12 atoms long (see page 585, Figure 2 legend). Ansari et al teach that the activation domains recruit elements of the endogenous transcriptional machinery to the promoter proximal to where the activator protein is bound to DNA (see page 583, left column, for example), which reads on a method of identifying a test compound that is known to modulate binding of natural transcription factors to the corresponding double stranded nucleic acid binding sites comprising providing a nucleic acid target and an anchor moiety covalently bonded to a flexible polyether linker covalently bonded to a test compound (VP1, VP2 or AH). It reads on contacting the conjugate-bound DNA sequence comprising an AdML promoter with transcriptional machinery and determining whether binding of a transcriptional

regulatory factor of the transcriptional machinery is modulated by the presence of a test compound.

Claims 1-4, 8-9, 11, 13, 17-18, 20, 31, 33, 35 and 37 are rejected under 35 U.S.C. 102(e) as being anticipated by Stanojevic (U.S. Patent Application Pub. No. 2003/105045, filed 3/14/2002).

Stanojevic teaches a method for assaying a test compound for activity as a transcriptional effector. Stanojevic teaches that the method includes linking the test compound covalently to a flexible linker domain which is covalently bound to a non-peptidic DNA binding domain to provide a test composition, the DNA (double stranded) binding domain having affinity for a DNA binding site on a DNA template sufficient to bind the site and to modulate transcription at a promoter, contacting the test composition with a transcription mixture including a DNA template, a eukaryotic RNA polymerase molecule capable of forming a complex with the test composition and the DNA template, a buffer and substrates under conditions suitable for RNA synthesis, such that RNA is synthesized and determining the quantity of RNA produced in the presence of the test composition compared to a level in the absence of the test composition, which is a measure of the activity of the test composition as a transcriptional modulator (see paragraph 0021, in particular). Stanojevic teaches that the DNA binding domain can be a nucleic acid or peptide nucleic acid or a sequence specific DNA binding natural product (see paragraphs 0013-0014). Stanojevic teaches that the DNA binding domain can also be a triplex forming oligonucleotide (TFO) that

binds in the major groove of the DNA helix (see paragraphs 0042-0045). Stanojevic teaches that the DNA binding domain can be a peptide analog such as a polyamide, or sequence specific DNA binding natural product such antibiotics or small organic moieties (see paragraphs 0046).

Stanojevic teaches that the DNA binding domain can be covalently linked to the effector DNA through a flexible linker (see paragraph 0059). Stanojevic teaches that the linker can be a polyglycol, or a plurality of units such as nucleotides, peptides, lower alkyls and other oxygen containing alkyl chain derivatives (see paragraph 0017), which reads on the claimed method wherein the claims linker is selected from the group of polypeptides, poly(ethyleneglycols), and C_{1-6} alkylenyl, alkenyl, alkene, alkyne and alkynyl. Stanojevic teaches that in at least some embodiments, the flexible linker has a length in the range of 10-100 Å or a range of 15-25 Å, or a range of 25-40 Å, or a range of 40-60 Å, or a range of 60-100 Å (see paragraph 0011, for example), which reads on the claimed method in which the linker moiety is at least 30 Å long. Stanojevic teaches that the natural transcription activator VP16 can be fused to GAL4 increased transcription *in vitro* (see paragraphs 0089-0090, for example). Stanojevic provides artificial transcription factors with transcription activity even greater than that of the natural transcription proteins (see paragraph 0040, for example).

Stanojevic teaches compositions comprising the artificial transcription factors comprising the test compound covalently attached to a flexible linker domain that is covalently bound to a non-peptidic DNA binding domain. Stanojevic also teaches a kit comprising a flexible linker covalently bound to a DNA binding domain and a reactive

end group that can be used to couple the construct to a test compound of interest to assess the activity of the composition. Stanojevic teaches that the kit would come with instructions for using the precursor compound in the disclosed methods (see paragraph 0076, for example), which reads on a kit for testing a compound's ability to modulate binding of a regulatory factor to a corresponding regulatory factor on a nucleic acid.

Claims 1, 3-4, 8-10, 13, 15, 17-19, 22, 26-28 and 31-32 are rejected under 35 U.S.C. 102(a) as being anticipated by Stanojevic and Young (Biochem 2002, Vol. 41 pages 7209-7216).

Stanojevic and Young teach a method for assaying a test compound for activity as a transcriptional effector to design potent synthetic transcriptional activators with activity that would be comparable to natural transcription factors (see page 7209, right column, 1st paragraph). Stanojevic and Young teach that the artificial transcription factor includes a DNA binding domain, which can be a triple-helix forming oligonucleotide (TFO) that binds in the major groove of the DNA helix, a composite linker moiety, and a short synthetic peptide activation domain (see page 7209, abstract, in particular). Stanojevic and Young teach that portions of various lengths from the strong natural transcription activator VP16 can be fused to GAL4 increased transcription *in vitro* and that the addition of a linker drastically increased the ability of the construct to increase transcription (see page 7211, left column, and page 7214, right column, for example). Stanojevic and Young teach that the combination of a long flexible polyglycol chain with a rigid nucleotide crosslinker appears to mimic the molecular geometry of natural

transcription factors (see page 7214, right column, 1st paragraph, for example), which reads on the linker moiety as an aptamer.

Stanojevic and Young teach that the method includes linking the VP16 lengths (test compounds) covalently to a flexible linker domain which is covalently bound to an DNA binding domain to provide a test composition, the DNA binding domain having affinity for a DNA binding site on a DNA template sufficient to bind the site and to modulate transcription at a promoter, contacting the test composition with a transcription mixture for *in vitro* transcription assays, such that the transcriptional activation in the presence of the test composition compared to a level in the absence of the test composition, which is a measure of the activity of the test composition as a transcriptional modulator (see page 7214, left column, Figure 5 in particular).

Stanojevic and Young teach compositions comprising the artificial transcription factors comprising the test compound covalently attached to a flexible linker domain that is covalently bound to a non-peptidic DNA binding domain. Stanojevic teaches that the DNA binding domain can be covalently linked to the effector DNA through a flexible polyglycol linker (see page 7211, left column and Figure 1, in particular), which reads on the claimed method wherein the claimed linker is selected from the group of polypeptides, poly(ethyleneglycols), and C_{1-6} alkylenyl, alkenyl, alkene, alkyne and alkynyl.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 11, 13, 20, 31 and 33 are rejected under 35 U.S.C. 103(a) as being obvious over Ansari et al (2001, Chem. Biol. Vol.8, pages 583-592, online pub. 5/8/2001) in view of Arora et al (J. Am. Chem. Soc. 2002, Vol 124, pages 13067-13071).

Applicant claims an *in vitro* method of evaluating one or more test compounds to identify test compounds that modulate binding of natural or artificial regulatory factors to corresponding single-, double-, or triple-stranded nucleic acid binding sites, the method comprising providing an isolated nucleic acid target conjugated or covalently bonded to an anchor moiety, a linker moiety covalently bonded to the anchor moiety, and a test compound bonded to the linker moiety, and then under transcription conditions, contacting *in vitro* the nucleic acid target of step to a reagent mixture comprising one or more natural or artificial regulatory factors specific for the binding site defined in the nucleic acid target, and then determining whether binding of the regulatory factor to the binding site defined in the nucleic acid target is modulated by presence of the test compound, wherein the linker moiety is at least 30 Å long.

The teaching of Ansari et al has been described in the above rejections. Ansari et al do not teach a method wherein the linker moiety is at least 30 Å long.

Arora et al teach artificial transcriptional activators comprised of a hairpin polyamide DNA binding domain and a peptide activation domain connected by flexible linkers of various length. Arora et al teach that the activation peptides used were AH and VP2, which have previously shown activation functionality when linked to a hairpin polyamide. Arora et al teach assays to determine the optimal length of the linker region in order to project the activating region away from the DNA in order to increase transcriptional activation. Arora et al teach transcription activators with linkers that have been increased in an incremental manner spanning 18, 27, 36 and 54 Å lengths. Arora et al et al teach that 36-45 Å is an optimal linking region length. Arora et al teach that the linker plays a role in determining the ability of the activating region to stimulate *in vitro* transcription and that the optimal spacing between the DNA and the activating region is 36-45 Å (see page 13068, right column, in particular). Arora et al teach that this knowledge is important in order to be able to design functioning transcription factors.

It would have been obvious to the skilled artisan to use a linker that is at least 30 Å in length in a method to identify test compounds that modulate the binding of transcriptional regulatory factors as taught by Ansari et al because Ansari et al teach substitution of a native protein dimerization domain with a various length flexible polylinker in order to modulate the function of a synthetic transcriptional activator (see page 584, left column, 3rd paragraph and page 587, left column, 2nd paragraph, for

example) and Arora et al et al teach that 36-45 Å is an optimal linking region length. The motivation to use a 36-45 Å (at least 30 Å) linking region is the expected benefit of optimized transcriptional activation over the transcriptional activation observed using constructs comprising linking region of shorter or longer length. There is a reasonable expectation of success in using a linking region that is at least 30 Å long to increase transcriptional activation since it has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 1, 3, 6-9, 13 and 15-18 are rejected under 35 U.S.C. 103(a) as being obvious over Ansari et al (2001, Chem. Biol. Vol.8, pages 583-592, online pub. 5/8/2001) in view of Ansari et al (2002, Curr. Opin. Chem. Biol. Vol. 6, pages 765-772).

Applicant claims an *in vitro* method of evaluating one or more test compounds to identify test compounds that modulate binding of natural or artificial regulatory factors to corresponding single-, double-, or triple-stranded nucleic acid binding sites, the method comprising providing an isolated nucleic acid target conjugated or covalently bonded to an anchor moiety, a linker moiety covalently bonded to the anchor moiety, and a test compound bonded to the linker moiety; and then under transcription conditions, contacting the nucleic acid target *in vitro* to a reagent mixture comprising one or more natural or artificial regulatory factors specific for the binding site defined in the nucleic acid target to determine whether binding of the regulatory factor to the binding site defined in the nucleic acid target is modulated by presence of the test compound,

wherein the anchor moiety is a major groove binding/ triple helix forming oligonucleotide, a peptide nucleic acid or a polyamide.

The teaching of Ansari et al (2001) has been described in the previous rejections.

Ansari et al (2001) do not teach a method comprising the use of an anchor moiety comprising a peptide nucleic acid (PNA) or a method comprising the use of a linker moiety comprising a bifunctional moiety consisting of polypeptides or polyethylene glycols.

Ansari et al (2002) teach the modular design of synthetic transcription factors in which the two functional modules (DNA binding domain and the regulatory domain) can be exchangeable (see page 765, right column for example). Ansari et al (2002) disclose efforts to replace naturally occurring modules with modules that can display programmable DNA targeting or regulatory activity. Ansari et al (2002) teach that protein DNA binding domains comprising zinc finger binding domains are used to generate binding constructs for specific DNA sequences (page 766, right column), Ansari et al (2002) also teach that triplex-forming oligonucleotide (TFOs) and peptide nucleic acids (PNA) have been used with some success to target specific DNA sequences and are exciting alternatives to peptide DNA binding domains in synthetic transcription factor design (see page 767, left column, 1st paragraph, for example). Ansari et al (2002) teach that the most frequently used DNA binding modules are TFO, PNA and polyamide domains (see page 768, Figure 3 legend, for example). Ansari et al (2002) also teach that the linker is an important variable in optimizing the transcriptional activating function of the construct (see page 769, left column). Ansari et al (2002) teach that while the

linker module for such constructs is usually a flexible or rigid peptide linked by covalent bonds, it can also be substituted with a linker derived from polyethylene glycol without loss of function (see page 768, Figure 3 legend, for example).

It would have been obvious to the skilled artisan to use a PNA as a DNA binding domain anchor in a construct comprising a linker domain and a test compound, such as a transcriptional activator domain, in the method taught by Ansari et al (2001), since Ansari et al (2001) teach that DNA binding motifs can be substituted into a linker/ activator construct in order to increase transcriptional activation and Ansari et al (2002) teach that PNA can be used successfully to target specific DNA sequences. The motivation to do so is the expected benefit of the ability to flexibly design modular transcriptional activators for specific purposes to allow greater control in regulating targeted genes. The development of ligand-responsive synthetic transcriptional activators with tunable potency is desirable to provide graded potency in response to physiological cues. It would also have been obvious to the skilled artisan to use a polyethylene glycol linker region in the construct because Ansari et al (2002) teach that it is a viable substitute for rigid or flexible peptides in transcriptional activator constructs. The motivation to use a polyethylene glycol linker would be the expected benefit of being able to vary the anchor, linker activator construct for specific use. There is a reasonable expectation of success to use PNA as a DNA binding domain or PEG as a linker since they have been used previously in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the

invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 22 and 29 are rejected under 35 U.S.C. 103(a) as being obvious over Ansari et al (2001, Chem. Biol. Vol.8, pages 583-592, online pub. 5/8/2001) as evidenced by Sadowski et al, in view of Arora et al (J. Am. Chem. Soc. 2002, Vol. 124, pages 13067-13071).

Applicant claims an *in vitro* method of evaluating one or more test compounds to identify test compounds that modulate binding of artificial regulatory factors to corresponding single-, double-, or triple-stranded nucleic acid binding sites, wherein the linker moiety is at least 30 Å long.

The teachings of Ansari et al and Sadowski et al have been discussed in the above rejection. Ansari et al and Sadowski et al do not teach the claimed method wherein the linker moiety is at least 30 Å long.

Arora et al teach artificial transcriptional activators comprised of a hairpin polyamide DNA binding domain and a peptide activation domain connected by flexible linkers of various length. Arora et al teach that the activation peptides used were AH and VP2. Arora et al teach transcription activators with linkers that have been increased in an incremental manner spanning 18, 27, 36 and 54 Å lengths. Arora et al et al teach that 36-45 Å is an optimal linking region length. Arora et al teach that the linker plays a role in determining the ability of the activating region to stimulate *in vitro* transcription and that the optimal spacing between the DNA and the activating region is 36-45 Å (see

page 13068, right column, in particular). Arora et al teach that this knowledge is important in order to be able to design functioning transcription factors.

It would have been obvious to the skilled artisan to use a linker that is at least 30 Å in length in a method to identify test compounds that modulate the binding of transcriptional regulatory factors as taught by Ansari et al because Ansari et al teach substitution of a native protein dimerization domain with a various length flexible polylinker in order to modulate the function of a synthetic transcriptional activator (see page 584, left column, 3rd paragraph and page 587, left column, 2nd paragraph, for example) and Arora et al et al teach that 36-45 Å is an optimal linking region length. The motivation to use a 36-45 Å (at least 30 Å) linking region is the expected benefit of optimized transcriptional activation over the transcriptional activation observed using constructs comprising linking region of shorter or longer length. There is a reasonable expectation of success in using a linking region that is at least 30 Å long to increase transcriptional activation since it has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 22 and 24-27 are rejected under 35 U.S.C. 103(a) as being obvious over Ansari et al (2001, Chem. Biol. Vol.8, pages 583-592, online pub. 5/8/2001) as evidenced by Sadowski et al (Nature, 1998, Vol. 335, pages 563-564) and in view of Ansari et al (2002, Curr. Opin. Chem. Biol., Vol. 6, pages 765-772).

Applicant claims an *in vitro* method of evaluating one or more test compounds to identify test compounds that modulate binding of natural or artificial regulatory factors to corresponding single-, double-, or triple-stranded nucleic acid binding sites, wherein the anchor moiety is a major groove binding/ triple helix forming oligonucleotide, a peptide nucleic acid or a polyamide.

The teachings of Ansari et al (2001) and Sadowski et al have been discussed in the previous rejections. Ansari et al (2001) and Sadowski et al do not teach the claimed method wherein the anchor moiety is a major groove binding/ triple helix forming oligonucleotide, a peptide nucleic acid or a polyamide or the linker moiety is a polyethylene glycol.

Ansari et al (2002) teach the modular design of synthetic transcription factors in which the two functional modules (DNA binding domain and the regulatory domain) can be exchangeable (see page 765, right column for example). Ansari et al (2002) disclose efforts to replace naturally occurring modules with modules that can display programmable DNA targeting or regulatory activity. Ansari et al (2002) teach that protein DNA binding domains comprising zinc finger binding domains are used to generate binding constructs for specific DNA sequences (page 766, right column), Ansari et al (2002) also teach that triplex-forming oligonucleotide (TFOs) and peptide nucleic acids (PNA) have been used with some success to target specific DNA sequences and are exciting alternatives to peptide DNA binding domains in synthetic transcription factor design (see page 767, left column, 1st paragraph, for example). Ansari et al (2002) teach that the most frequently used DNA binding modules are TFO, PNA and polyamide

domains (see page 768, Figure 3 legend, for example). Ansari et al (2002) also teach that the linker is an important variable in optimizing the transcriptional activating function of the construct (see page 769, left column). Ansari et al (2002) teach that while the linker module for such constructs is usually a flexible or rigid peptide linked by covalent bonds, it can also be substituted with a linker derived from polyethylene glycol without loss of function (see page 768, Figure 3 legend, for example). Ansari et al (2002) teach that the linker is an important variable in optimizing the transcriptional activating function of the construct (see page 769, left column).

It would have been obvious to the skilled artisan to use a PNA as a DNA binding domain anchor in a construct comprising a linker domain and a test compound, such as a transcriptional activator domain, in the method taught by Ansari et al (2001), since Ansari et al (2001) teach that DNA binding motifs can be substituted into a linker/ activator construct in order to increase transcriptional activation and Ansari et al (2002) teach that PNA can be used successfully to target specific DNA sequences. The motivation to do so is the expected benefit of the ability to flexibly design modular transcriptional activators for specific purposes to allow greater control in regulating targeted genes. The development of ligand-responsive synthetic transcriptional activators with tunable potency is desirable to provide graded potency in response to physiological cues. It would also have been obvious to the skilled artisan to use a polyethylene glycol linker region in the construct because Ansari et al (2002) teach that it is a viable substitute for rigid or flexible peptides in transcriptional activator constructs.

The motivation to use a polyethylene glycol linker would be the expected benefit of being able to vary the anchor, linker activator construct for specific use. There is a reasonable expectation of success to use PNA as a DNA binding domain or PEG as a linker since they have been used previously in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 1, 3, 8-9, 11, 13, 17-18, 20, 31 and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Felgner et al (U.S. Patent No. 6,165,720) in view of Arora et al (J. Am. Chem. Soc. 2002, Vol. 124, pages 13067-13071).

Applicant claims an *in vitro* method of evaluating one or more test compounds to identify test compounds that modulate binding of natural or artificial regulatory factors to corresponding single-, double-, or triple-stranded nucleic acid binding sites, wherein the anchor moiety is a major groove binding/ triple helix forming oligonucleotide, a peptide nucleic acid or a polyamide. Applicant also claims a composition of matter comprising an isolated nucleic acid target that defines a desired or putative binding site for a regulatory factor, the isolated nucleic acid target having covalently bonded thereto, at a point proximate to the binding site an anchor moiety, a linker moiety covalently bonded to the anchor moiety, and a test compound conjugated to the linker moiety.

Felgner et al teach a PNA clamp in which two PNA sequences are joined by a hairpin linker that can hybridize to a single and double stranded nucleic acids (see column 2, lines 15-40, for example). Felgner et al teach that the PNA can be conjugated to a transcriptional activator domain peptide (see column 3, lines 37-45, for example. Felgner et al teach that the PNA conjugates can be used as artificial transcription promoters (see column 2, lines 41-55, for example). Felgner et al teach a method to screen compounds which activate transcription comprised of linking a compound to a PNA, hybridizing it to a plasmid containing a reporter gene and then determining the level of expression of the reporter gene, where and increase in expression in the presence of the compound indicates that the compound is an activator of transcription (see column 4, lines 52-6,7 for example). Felgner et al teach that endogenous transcription factors are proteins that contain two domains, the DNA binding domain and the transcription activation domain. The DNA binding domain usually binds to specific DNA sequences located in the enhancer region and brings the transcription activation domain into proximity of the minimal promoter where it interacts with RNA polymerase to activate transcription. Felgner et al teach that the DNA-bound transcription factors interact with the transcription complex and increase the transcription rate. Felgner et al teach that transcription activator peptides contemplated for use with the PNA DNA binding construct include VP16, P65 and OCT-2 (see column 8, lines 12-59, for example). Felgner et al teach that test compound can be coupled to PNA using standard crosslinking methods using crosslinking agents such as heterodifunctional crosslinkers with two or more reactive groups that allow for sequential conjugations

(see column 9, lines 15-30, column 12, lines 10-30 and Example 12, column 22, lines 1-45, for example). Felgner et al teach a direct crosslink between the PNA and the activator peptides (see column 22, lines 6-10, for example).

Felgner et al does not teach a linker moiety bonded to the anchor moiety.

Arora et al teach artificial transcriptional activators comprised of a hairpin polyamide DNA binding domain and a peptide activation domain connected by flexible linkers of various length. Arora et al teach that the activation peptides used were AH and VP2 (a derivative of VP16), which have previously shown activation functionality when linked to a hairpin polyamide. Arora et al teach assays to determine the optimal length of the linker region comprised of proline residues or polyethylene glycols, in order to project the activating region away from the DNA in order to increase transcriptional activation. Arora et al teach transcription activators with linkers that have been increased in an incremental manner spanning 18, 27, 36 and 54 Å lengths. Arora et al et al teach that 36-45 Å is an optimal linking region length. Arora et al teach that the linker plays a role in determining the ability of the activating region to stimulate *in vitro* transcription and that the optimal spacing between the DNA and the activating region is 36-45 Å (see page 13068, right column, in particular). Arora et al teach that this knowledge is important in order to be able to design functioning transcription factors.

It would have been obvious to the skilled artisan to use a linker that is at least 30 Å in length in a method to identify test compounds that modulate the binding of transcriptional regulatory factors as taught by Felgner et al because Felgner et al teach that a variety of crosslinking agents can be used to conjugate any desired chemical

group to the PNA (see column 12, lines 15-35, for example) and Arora et al et al teach that 36-45 Å is an optimal linking region length. The motivation to use a 36-45 Å (at least 30 Å) linking region is the expected benefit of optimized transcriptional activation over the transcriptional activation observed using constructs comprising linking region of shorter or longer length. There is a reasonable expectation of success in using a linking region that is at least 30 Å long to increase transcriptional activation since it has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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9/15/2006

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